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Intragraft TGF-β_1 mRNA: A correlate of interstitial fibrosis and chronic allograft nephropathy

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Intragraft TGF-β_1 mRNA: A correlate of interstitial fibrosis and chronic allograft nephropathy. Chronic allograft nephropathy is a relentlessly progressive process and a major cause of long-term graft dysfunction and ultimate failure. Interstitial fibrosis, tubular atrophy, and glomerular and vascular lesions characterize this mechanistically unresolved disorder. Given the prominent role of TGF-β_1 in tissue repair and in fibrosis, we have explored the hypothesis that fibrosis and chronic allograft nephropathy would be distinguished by intragraft TGF-β_1 mRNA expression. This postulate was tested by mRNA phenotyping of RNA isolated from 127 human renal allograft biopsies. Reverse transcription assisted polymerase chain reaction was used to amplify and identify ingraft gene expression. Our investigation demonstrated a significant correlation between intragraft TGF-β_1 mRNA display and renal allograft interstitial fibrosis and chronic allograft nephropathy. In contrast, intragraft expression of mRNA encoding immunoregulatory cytokines, IL-2, IFN-γ, IL-4, IL-10, or cytotoxic attack molecules, granzyme B and perforin was not a correlate of interstitial fibrosis or chronic allograft nephropathy. Our studies identify, for the first time, a significant association between intragraft TGF-β_1 mRNA expression and renal allograft interstitial fibrosis, and advance a candidate molecular mechanism for chronic allograft nephropathy.

Renal transplantation is the treatment of choice for most patients afflicted with end-stage renal disease. A steady improvement in the outcome of renal allografts has been noted, especially those of cadaveric origin and those involving second or subsequent transplantations. In 1988, the one year graft survival rate among 6073 primary, 954 second, and 173 subsequent cadaveric grafts, was 77.4%, 69.4% and 58.5%, respectively; in 1993, the rate had improved to 83.9% for 6,991 primary grafts, 81.2% for 855 second grafts, and 72.6% for 151 subsequent grafts [1]. Improved understanding of the anti-allograft response, refined usage of cyclosporine (CsA), selective application of monoclonal or polyclonal antibodies, and the routine use of infection prophylaxis protocols have all contributed to the gratifying short-term results reported following renal transplantation.

The advances in the treatment strategies, however, have had modest impact on the long-term outcome following renal transplantation. The half-life (the time needed for 50% of the grafts functioning 1 year after transplantation to fail) of cadaveric renal allografts has increased from about eight years in 1988 to only about 10 years in 1992 [1]. The most common cause of failure of long-term allografts, chronic allograft rejection/nephropathy [2–4], remains poorly understood with respect to underlying molecular and immunologic mechanism(s). Antigen specific cellular and humoral immunity, additional cellular elements, including macrophages and platelets (and their secretory products, eicosanoids), and non-immune factors such as dyslipidemia and hypertension have all been hypothesized as pathogenic factors [2–4].

Histopathological features associated with progressive allograft dysfunction have been described [5, 6]. The pertinent morphological alterations include interstitial fibrosis, increased extracellular matrix accumulation, and glomerular and vascular lesions.

In this investigation, we have explored the hypothesis that intragraft expression of transforming growth factor-β_1 (TGF-β_1) is a correlate of interstitial fibrosis and chronic allograft nephropathy in human renal allografts. TGF-β_1, a 25 kDa homodimeric secretory product of many cell lines including platelets, T cells, mesangial cells and monocytes/macrophages, is a multifunctional cytokine [reviewed in 7, 8]. It is a prominent member of the cytokine cascade involved in tissue repair, and considerable data support its fibrogenic properties. Some of the biological properties of this pluripotent cytokine include its ability to promote the production of extracellular matrix materials (fibronectin, collagens and proteoglycans), enhance the expression of integrins, and reduce the activities of matrix degrading proteases [7, 8].

The data from this investigation of intragraft gene expression in human renal allograft recipients demonstrate a significant correlation between intragraft expression of TGF-β_1 mRNA and interstitial fibrosis and chronic allograft nephropathy. Moreover, the specificity of this association is emphasized by the lack of correlation between histologic features of either interstitial fibrosis or chronic allograft nephropathy and intragraft display of type I cytokines (IL-2, IFN-γ), type II cytokines (IL-4 and IL-10), and cytotoxic attack molecules (granzyme B and perforin).

Methods

Subjects

One hundred twenty-seven renal allograft core tissue samples were obtained from 107 patients who received their renal allografts at our institutions, The New York Hospital (New York City, NY, USA; $N = 71$), or Hennepin County Medical Center

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(Minneapolis, MN, USA; $N = 36$). Eighty-eight patients underwent one biopsy each; 18 recipients, two biopsies, and one patient underwent three renal biopsies. The biopsies were performed for clinical reasons to determine the basis for graft dysfunction. The renal allograft recipients were managed with an immunosuppressive regimen of antilymphocyte antibodies and/or cyclosporine, prednisone and azathioprine [9].

Histopathological evaluation of renal allograft biopsy specimens

The biopsy tissues included in this study of intragraft gene expression were classified on the basis of the Banff working classification criteria [6]. The tissue samples, in each instance, were considered adequate for diagnostic purposes; at least three slides stained with H and E, and four samples with special stains (PAS, trichrome, Azan and silver stain) were examined [6]. The histological classification of the biopsy specimen was made without the knowledge of the results of the molecular studies. The following criteria and grading were used for the histopathological diagnosis of renal allograft biopsy specimens.

Interstitial fibrosis and tubular atrophy. The following features were utilized and were considered independent of possible etiology of interstitial fibrosis (such as chronic rejection, chronic CsA toxicity and/or chronic ischemic injury). The separation of the tubules by increased interstitial connective tissue and fibrosis was observed and confirmed by Azan or trichrome stain. Interstitial fibrosis was graded on a scale of 0, 1, 2, and 3, based on the severity of the observed lesion [6]. The tubules showing wrinkling and thickening of the tubular basement membrane were detected with the PAS stain and the degree of tubular atrophy was also graded on a scale of 0, 1, 2, and 3, as proposed in the Banff classification [6]. The extent of fibrosis usually paralleled that of tubular atrophy.

Chronic allograft nephropathy. The histologic diagnosis was based on the presence of interstitial fibrosis and tubular atrophy and additional vascular changes including arterial fibrous intimal thickening, and glomerular changes such as glomerular tuft shrinkage, sclerosis and thickening or wrinkling of the basement membrane. The glomerular changes, interstitial fibrosis, tubular atrophy and vascular changes were graded on a scale of 0, 1, 2 and 3, as proposed [6].

Acute rejection. The histologic diagnosis of acute rejection was based on the presence of significant interstitial infiltration by lymphocytes, and invasion of tubules (tubulitis) and/or the walls of blood vessels (intimal arteritis) [6].

CsA-associated changes. Isometric vacuolization of tubules, eosinophilic inclusions, microcalcification, hyaline arteriolar thickening and striped or patch fibrosis were identified by light microscopy and considered as features of CsA associated changes [6].

Molecular studies of renal allograft biopsy specimens: RNA isolation

Portions of renal biopsy tissue were snap-frozen in liquid nitrogen and stored at −70°C for later RNA extraction. The renal tissue was homogenized with a tissue tearer in 4 M guanidinium isothiocyanate solution and loaded on top of 5.7 M CsCl cushion and centrifuged at 20°C and 50,000 rpm for 16 to 20 hours using a TLS 55 rotor in a Beckmann TL100 ultracentrifuge. RNA was isolated as described previously [10] and quantified by spectrophotometry.

Reverse transcription assisted PCR. Reverse transcription assisted PCR was done as previously described [10]. In brief, 1 μg of total RNA was reverse-transcribed into cDNA in a 20-μl reaction mixture containing 50 mM Tris-HCl (pH 8.3); 75 mM KCl; 10 mM DTT; 3 mM $MgCl_2$; 200 units of Moloney murine leukemia virus reverse transcriptase, 100 ng/reaction of random hexanucleotide primers, and 0.5 mM each of deoxyribonucleoside triphosphatases (dNTPs): dATP, dCTP, dGTP, and dTTP. The reaction mixtures were incubated at 37°C for one hour and then incubated at 65°C for 10 minutes to deactivate the reverse transcriptase. The final volume was adjusted to 50 μl with TE buffer (pH 8.0) and the cDNAs were stored at −20°C till further analysis. A 3 μl of cDNA from a total of 50 μl was amplified with 1 unit of *Taq* DNA polymerase in a final volume of 50 μl containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 40 μM of each dNTP, and 0.2 μM of each primer. Thirty-five cycles of amplification were carried out in a thermocycler (Gene Amp PCR System 9600, Perkin Elmer, CA, USA), and the amplification profile was as follows: denaturing at 94°C for 30 seconds, primer annealing at 57°C for 30 seconds, and primer extension at 72°C for 30 seconds. Before cycling, the samples were preheated at 94°C for 30 seconds, and after amplification an extra five minutes were added for extension at 72°C. Aliquots (8 μl) of each resulting PCR product were applied to a 2% NuSieve agarose/1% agarose gel, resolved by electrophoresis, visualized by ethidium bromide staining, and photographed.

Design and synthesis of sequence specific oligonucleotide primers

Table 1 lists the sequence of the oligonucleotide primers used to amplify mRNA encoding cytokines, cytotoxic attack molecules, and the constitutively expressed β-actin mRNA. The predicted size of the PCR product generated with a particular primer pair is also listed in Table 1. The primers were designed to flank an intron site in the target sequence. This primer design permits detection of genomic contamination of the RNA preparation from the renal biopsy specimens. Also, in each instance, β-actin mRNA was amplified as an internal control for reverse transcription assisted PCR [10].

Statistical analysis

Fisher's Exact Test for 2×2 tables was used to assess the correlation between intragraft mRNA expression and histopathological diagnosis. Sensitivity, specificity, and positive and negative predictive values were also calculated when a statistically significant correlation ($P < 0.05$) between intragraft mRNA expression and a specific renal histological diagnosis was observed. Mann-Whitney two sample test was used to determine two-tailed P values following comparison of the mean scores of glomerular, vascular, tubular changes and the severity of interstitial fibrosis in biopsies distinguished by the presence or absence of intragraft mRNA expression.

Results

Histological classification of renal allograft biopsies

Figure 1 illustrates the histological diagnosis of renal biopsies classified on the basis of Banff working classification criteria [6]. Among the 127 biopsies, 67 were classified as acute rejection, 20 as chronic allograft nephropathy, 16 as chronic allograft nephropathy and acute rejection, 13 as CsA associated changes. Among

Table 1. Sequences of oligonucleotide primers used in RT-PCR

TGF-β_1	(*Nature* 316:701–705, 1985)		
5′Sense	CTG CGG ATC TCT GTG TCA TT	(2154-2173)	246 bp
3′Antisense	CTC AGA GTG TTG CTA TGG TG	(2380-2399)	
IL-2	(*PNAS* 80: 7437–7441, 1983)		
5′Sense	CCT CTG GAG GAA GTG CTA AA	(3113-3132)	149 bp
3′Antisense	ATG GTT GCT GTC TCA TCA GC	(5087-5106)	
IFN-γ	(*Nature* 298:859–863, 1982)		
5′Sense	GCA GGT CAT TCA GAT GTA GC	(1831-1850)	177 bp
3′Antisense	TTC CTT GAT GGT CTC CAC AC	(2083-2102)	
IL-4	(*PNAS* 83:5894–5898, 1986)		
5′Sense	TTC TAC AGC CAC CAT GAG AAG	(298-318)	227 bp
3′Antisense	CAG CTC GAA CAC TTT GAA TAT	(504-524)	
IL-10	(*PNAS* 88:1172–1176, 1991)		
5′Sense	TAA GGG TTA CCT GGG TTG CCA A	(252-273)	257 bp
3′Antisense	CAC TCA TGG CTT TGT AGA TGC C	(487-508)	
Perforin	(*Immunogenetics* 30:452–457, 1989)		
5′Sense	CAG TAC AGC TTC AGC ACT GAC	(496-516)	176 bp
3′Antisense	ATG AAG TGG GTG CCG TAG TTG	(651-671)	
Granzyme B	(*Gene* 87:265–271, 1990)		
5′Sense	TGC AGG AAG ATC GAA AGT GCG	(3360-3380)	180 bp
3′Antisense	GAG GCA TGC CAT TGT TTC GTC	(4163-4183)	
β-Actin	(*PNAS* 82:6133–6137, 1985)		
5′Sense	GGT CAC CCA CAC TGT GCC CAT	(2139-2159)	350 bp
3′Antisense	GGA TGC CAC AGG ACT CCA TGC	(2563-2583)	

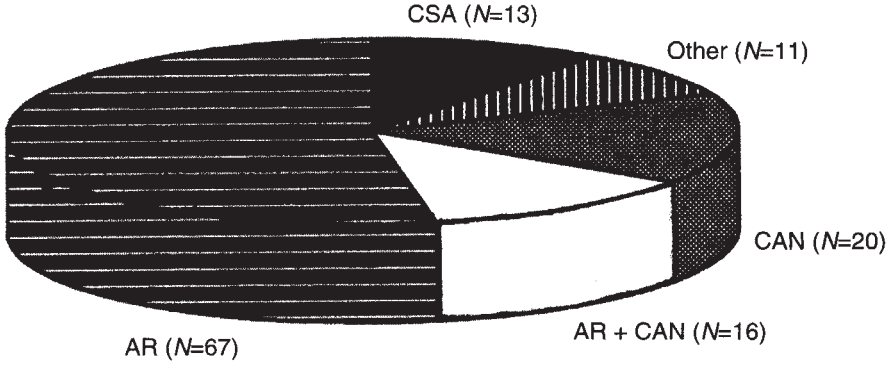

Fig. 1. *Histopathological diagnosis of renal allograft biopsy specimens.* The histological diagnosis of renal allograft biopsy samples were based on Banff working classification criteria for diagnosing kidney transplant pathology [6]. Abbreviations are: AR, acute rejection (N = 67); AR + CAN, acute rejection plus chronic allograft nephropathy (N = 16); CAN, chronic allograft nephropathy (N = 20); CsA, CsA associated changes (N = 13); Other, non-specific changes (N = 2); ATN (N = 2); normal (N = 2), recurrent renal disease (N = 4), and *de novo* glomerular disease (N = 1). The numbers (N) represent the number of tissue samples.

the 36 biopsies classified as chronic allograft nephropathy, 23 displayed features of transplant glomerulopathy [6], and among 13 biopsies classified as CsA associated changes, 11 displayed features of arteriolopathy [6]. Of the 11 renal biopsies classified as other, 4 expressed histological features of recurrent renal disease; 2 revealed non-specific morphological alterations, 2, ATN, 2, normal, and one was *de novo* glomerular disease (Fig. 1).

Interstitial fibrosis was observed in 87 of the 127 biopsy specimens. Table 2 lists the histological diagnosis of the biopsies distinguished on the basis of presence or absence of interstitial fibrosis.

Table 2. Histological diagnosis of renal allograft biopsies distinguished by the presence or absence of interstitial fibrosis

Interstitial fibrosis	Histological Classification				
	AR	AR + CAN	CAN	CsA	Other
Present (N = 87)	35	16	20	10	6
Absent (N = 40)	32	0	0	3	5

Abbreviations are: AR, acute rejection; CAN, chronic allograft nephropathy; CsA, cyclosporine associated morphological changes.

Intragraft TGF-β_1 mRNA expression in renal allograft interstitial fibrosis and chronic allograft nephropathy

TGF-β_1 is a potent fibrogenic cytokine with a prominent role in tissue repair following injury. This pluripotent cytokine induces an increase in extracellular matrix materials [7, 8]. Given that allografts are targets of multiple insults (ischemia, rejection and drugs) and given the prominent feature of fibrosis in chronic allograft nephropathy [6], we determined the correlation between intragraft TGF-β_1 mRNA expression and renal allograft interstitial fibrosis and chronic allograft nephropathy.

Table 3 shows the significant correlation between intragraft expression of intragraft TGF-β_1 mRNA and interstitial fibrosis. The association between intragraft TGF-β_1 mRNA expression and interstitial fibrosis was significant at P = 0.03 by Fischer's Exact Test.

Additional analysis of the correlation revealed that the sensitivity of intragraft TGF-β_1 mRNA expression was 0.61 and the

Table 3. Correlation between intragraft mRNA expression and renal allograft interstitial fibrosis

Intragraft mRNA[a]		Renal allograft fibrosis + (N = 87)	Renal allograft fibrosis − (N = 40)	P[b]
TGF-β_1 mRNA	+ (69)	53	16	
	− (58)	34	24	0.03
IL-2 mRNA	+ (10)	7	3	
	− (117)	80	37	1.00
IFNγ mRNA	+ (56)	38	18	
	− (66)	44	22	0.99
IL-4 mRNA	+ (2)	2	0	
	− (85)	55	30	0.54
IL-10 mRNA	+ (55)	36	19	
	− (72)	51	21	0.56
Granzyme B mRNA	+ (76)	50	26	
	− (51)	37	14	0.44
Perforin mRNA	+ (49)	38	11	
	− (77)	48	29	0.08

[a] Total RNA was isolated and reverse transcribed into cDNA and amplified by PCR with the sequence specific primer pairs listed in Table 1
[b] Two-tailed P value derived with Fischer's Exact Test for 2 × 2 tables

Table 4. Correlation between intragraft mRNA expression and chronic allograft nephropathy

Intragraft mRNA[a]		Chronic allograft nephropathy + (N = 36)	Chronic allograft nephropathy − (N = 91)	P[b]
TGF-β_1 mRNA	+ (69)	26	43	
	− (58)	10	48	0.01
IL-2 mRNA	+ (10)	2	8	
	− (117)	34	83	0.72
IFNγ mRNA	+ (56)	15	41	
	− (66)	19	47	0.84
IL-4 mRNA	+ (2)	0	2	
	− (85)	22	63	0.61
IL-10 mRNA	+ (55)	12	43	
	− (72)	24	48	0.16
Granzyme B mRNA	+ (76)	19	57	
	− (51)	17	34	0.32
Perforin mRNA	+ (49)	17	32	
	− (77)	19	58	0.31

[a] Total RNA was isolated and reverse transcribed into cDNA and amplified by PCR with the sequence specific primer pairs listed in Table 1
[b] Two-tailed P value derived with Fischer's Exact Test for 2 × 2 tables

specificity was 0.60. The positive predictive value was 0.77 and the negative predictive value was 0.41.

Among the 87 biopsies distinguished by the presence of interstitial fibrosis, 36 biopsies displayed histopathological features of chronic allograft nephropathy. Twenty-six of the 36 biopsies classified as chronic allograft nephropathy were positive for intragraft TGF-β_1 mRNA expression. The significant correlation (P = 0.01, Fischer's Exact Test) between intragraft TGF-β_1 mRNA expression and chronic allograft nephropathy is shown in Table 4.

Additional evaluation of the correlation between intragraft TGF-β_1 mRNA expression and chronic allograft nephropathy revealed that the sensitivity of intragraft TGF-β_1 mRNA expression was 0.72, and the specificity was 0.53. The positive predictive value was 0.38, and the negative predictive value was 0.83.

Quantitative assessment of morphological changes revealed significant differences between the intragraft TGF-β_1 mRNA positive group and theTGF-β_1 mRNA negative group with respect to severity of interstitial fibrosis and tubular changes. The mean ± SEM score of interstitial fibrosis in the TGF-β_1 mRNA positive group was 1.33 ± 0.13 (N = 69 biopsies) and was 0.94 ± 0.13 (N = 58) in the TGF-β_1 mRNA negative group; the difference was significant at P = 0.04 by Mann-Whitney two sample test. The mean ± SEM score of tubular atrophy in the TGF-β_1 mRNA positive group was 1.18 ± 0.12, and in the negative group, 0.77 ± 0.10 (P = 0.03). The mean scores for the chronic glomerular changes, and for the chronic vascular changes were also higher in the intragraft TGF-β_1 mRNA positive group compared to the negative group (chronic glomerular changes, 0.59 ± 0.11 vs. 0.36 ± 0.10; and chronic vascular changes, 0.55 ± 0.10 vs. 0.44 ± 0.11). These differences, however, were not statistically significant by Mann-Whitney two-sample test.

Intragraft expression of IL-2 mRNA or IFN-γ mRNA in interstitial fibrosis and chronic allograft nephropathy

IL-2 is a prototypic T-cell growth factor that promotes T-cell immune responses (such as cytotoxic T cell generation [11]), considered detrimental to the well-functioning of allografts. IFN-γ is a proinflammatory polypeptide that enhances not only immune effector mechanisms but also the display of HLA-proteins, the primary targets of anti-allograft responses [12]. IL-2 and IFN-γ are products of T helper type 1 cells. We [13] and others [14] have reported that intragraft expression of IL-2 mRNA is a correlate of acute rejection of human renal allografts. Given the prominent role of these cytokines in host immune assault responses, we sought to determine whether intragraft expression of IL-2 mRNA or IFN-γ mRNA is also a correlate of allograft interstitial fibrosis and chronic allograft nephropathy.

Table 3 shows that intragraft expression of IL-2 mRNA or IFN-γ mRNA is not a significant correlate of renal interstitial fibrosis. The correlation between intragraft expression of these proinflammatory cytokines and chronic allograft nephropathy is also not significant (Table 4).

Intragraft expression of IL-10 mRNA or IL-4 mRNA in interstitial fibrosis and chronic allograft nephropathy

IL-10, originally designated as cytokine synthesis inhibitory factor, has multiple effects on the immune system, including promotion of B-cell dependent immunity [15]. Indeed, in our earlier study of intragraft gene expression, a significant correlation was found between intragraft IL-10 mRNA expression and acute rejection [13]. Since chronic allograft nephropathy/rejection might be a consequence of repetitive immune injury, we determined whether intragraft expression of IL-10 mRNA is a correlate of interstitial fibrosis and/or chronic allograft nephropathy. We also determined whether IL-4, a multifunctional cytokine with a prominent role in immune regulation [16], is a molecular correlate of renal allograft interstitial fibrosis and/or chronic allograft nephropathy.

Results found by amplifying the IL-10 mRNA or IL-4 mRNA by reverse transcription assisted PCR are shown in Tables 3 and 4. It is evident that neither the intragraft expression of IL-10

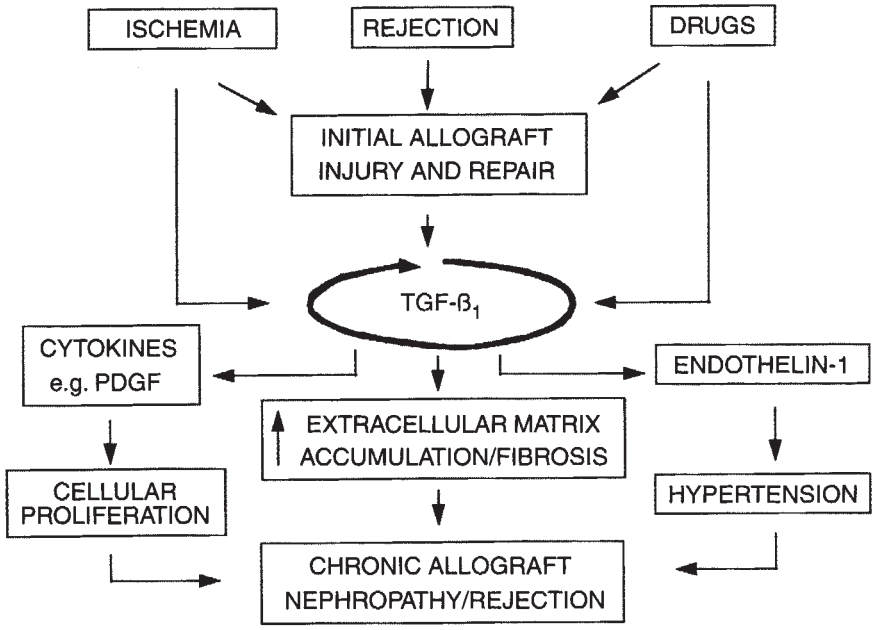

Fig. 2. *Schema for the pathogenesis of chronic allograft nephropathy/rejection.* In this fibrogenic cytokine hypothesis, intrarenal expression of TGF-β_1 represents a significant pathogenetic molecular event for the expression of chronic allograft nephropathy/rejection. The ability of TGF-β_1 to promote its own production (positive feedback loop), and its additional properties (such as regulation of PDGF activity, stimulation of endothelin-1 production) are also incorporated into this framework. That two of the most commonly used drugs in transplantation, CsA and steroids, stimulate TGF-β_1 expression is also considered in this formulation regarding the cascade of events involved in chronic allograft nephropathy/rejection.

mRNA nor the intragraft expression of IL-4 mRNA is a significant correlate of either renal allograft interstitial fibrosis (Table 3) or chronic allograft nephropathy (Table 4).

Intragraft expression of granzyme B mRNA or perforin mRNA in interstitial fibrosis and chronic allograft nephropathy

Elegant studies have demonstrated that intragraft expression of CTL-specific serine protease, granzyme B, is a significant correlate of human renal allograft rejection [17]. Data also exist to show that the pore-forming protein, perforin, is a major participant in the lytic machinery of CTL [18]. Recent studies have also emphasized the importance of an earlier acute rejection episode to subsequent occurrence of chronic rejection [19]. The possibility thus exists that chronic allograft nephropathy/rejection might be due to CTL-dependent cellular effector mechanism(s). In light of these considerations, we determined whether intragraft expression of granzyme B mRNA or perforin mRNA is a molecular correlate of allograft fibrosis and/or chronic allograft nephropathy.

Results found by amplifying cDNA with a granzyme B sequence specific primer pair or a perforin sequence specific primer pair, are shown in Tables 3 and 4. Unlike the significant correlation found between intragraft TGF-β_1 mRNA expression and renal interstitial fibrosis and chronic allograft nephropathy, and in a fashion similar to the lack of correlation between intragraft expression of mRNA encoding IL-2, IFN-γ, IL-4 or IL-10 and allograft fibrosis or chronic allograft nephropathy, intragraft expression of granzyme B mRNA or perforin mRNA was not a correlate of either renal allograft interstitial fibrosis (Table 3) or chronic allograft nephropathy (Table 4).

Discussion

The new finding that has emerged from this investigation is that intragraft expression of mRNA encoding TGF-β_1 is a significant correlate of human renal allograft interstitial fibrosis and chronic allograft nephropathy. This original observation also emphasizes the specificity of the association between intragraft TGF-β_1 mRNA display and graft fibrosis and chronic allograft nephropathy. Whereas intragraft TGF-β_1 mRNA expression is significantly associated with the fibrosis and chronic allograft nephropathy, other immune regulatory cytokines (IL-2, IFN-γ, IL-4 and IL-10) and potent mediators of cytotoxicity (granzyme B and perforin) are not significant correlates.

Significance of intragraft TGF-β_1 mRNA expression

A number of well-characterized biological properties of TGF-β_1 support the hypothesis that intragraft expression of TGF-β_1 might be mechanistically linked to the fibrotic process observed in the allograft. The relevant activities include: (a) stimulation of the production of extracellular matrix materials, fibronectin, collagens and proteoglycans [7, 8]; (b) promotion of the production of protease inhibitors such as plasminogen activator inhibitor type 1; (c) enhancement of the expression of matrix-anchoring proteins such as integrins; and (d) reduction in the activity of matrix-degrading proteins such as plasminogen activator [7, 8].

Evidence that TGF-β_1 engenders in vivo renal disease and fibrosis

In a rodent model of glomerulonephritis induced with a single injection of antithymocyte serum, intrarenal expression of TGF-β was found to be associated with increased extracellular matrix accumulation; also, anti-TGF-β antibodies or proteoglycans (natural regulators of TGF-β activity) constrained the production and accumulation of extracellular matrix material in this rodent model [8]. In the multiple injection model, sustained intrarenal expression of TGF-β_1 was observed and such persistent expression was associated with tubulointerstitial fibrosis and glomerulosclerosis [8]. Direct evidence that TGF-β_1 induces renal sclerosis has also been obtained. Isaka et al [20] have demonstrated that *in vivo* transfection of TGF-β_1 gene into the rat kidney results in

glomerulosclerosis. In this elegant study, transfection of the gene encoding platelet-derived growth factor resulted in the proliferation of glomerular cells only, and not in a major increase in extracellular matrix materials. Experimental data from rats made diabetic with the islet cell toxin streptozocin [8], and clinical data from diabetic patients with renal disease or mesangial proliferative glomerulonephritis, also support the hypothesis that TGF-β_1 is associated with increased extracellular matrix accumulation and fibrosis. In this regard, it is noteworthy that the expression of TGF-β_1 is stimulated by hyperglycemia [21] as well as by angiotensin II (Ang II) [22], and that the strict control of blood glucose or the inhibition of Ang II generation retards the progression of diabetic renal disease [23, 24].

Mechanistic and clinical implications of intragraft TGF-β_1 mRNA expression

Our data, viewed in the context of earlier data that TGF-β_1 participates in the expression of experimental or clinical renal disease states characterized by fibrosis, provide a molecular mechanism for the interstitial fibrosis associated with chronic allograft rejection/nephropathy. As schematically shown in Figure 2, it can be hypothesized that allograft injury results in TGF-β_1 expression, given its prominent role in tissue repair. TGF-β_1 then engenders interstitial fibrosis by virtue of its ability to promote extracellular matrix material accumulation and fibroblast proliferation. In addition to ischemia and rejection causing allograft injury, immunosuppressive therapy might also contribute to allograft injury since CsA [10] as well as steroids [25] stimulate TGF-β_1 expression. This unique effect of these two drugs widely used in clinical transplantation, coupled with our demonstration that the cytokines whose expression is suppressed by CsA or steroids (such as IL-2, IFN-γ) are not correlates of interstitial fibrosis and chronic allograft nephropathy, provides an explanation for the lack of a therapeutic benefit observed with CsA or steroids in the management of chronic allograft nephropathy/rejection.

TGF-β_1 stimulates its own production via specific response elements located in the TGF-β_1 promoter region [26]. Thus, a self-perpetuating process contributing to further progression of renal lesions can be entertained. Additional cytokine/polypeptide regulated mechanisms can also be considered. For example, TGF-β_1 regulates not only the expression of cytokines like PDGF and fibroblast growth factor but also that of the potent vasoconstrictor, endothelin-1 [27]. This cascade of events, viewed in the context of the progressive nature of renal disease due to intrarenal hemodynamic mechanisms [28], provides a conceptual framework for the relentlessly progressive nature of chronic allograft rejection/nephropathy.

Elegant experimental data exist that antibodies directed at the TGF-β protein or the proteoglycans prevent TGF-β-associated renal disease [8]. We have constructed a novel deoxynucleotide antigene oligomer complementary to the TGF-β_1 promoter DNA that prevents TGF-β_1 expression [29]. The new observation from the current investigation, that intragraft TGF-β_1 expression is a molecular correlate of renal allograft interstitial fibrosis and chronic allograft nephropathy, considered with the paucity of effective therapy for chronic rejection, suggests that the above-mentioned strategies might be of therapeutic value in preventing the development of and/or constraining the progression of chronic allograft nephropathy.

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